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(54) Title: USE OF THYMOSIN ALPHA 1 FOR THE MANUFACTURE OF A MEDICAMENT FOR PROMOTING STEM CELL DEVELOPMENT

(57) Abstract

A method of promoting stem cell development in a mammal in need of stem cell development includes administering to the mammal a stem cell development enhancing effective amount of Thymosin α_1 ($T\alpha_1$).

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USE OF THYMOSIN ALPHA 1 FOR THE MANUFACTURE OF A MEDICAMENT FOR PROMOTING STEM CELL DEVELOPMENT

Field of the Invention

The present invention relates generally to a method of promoting stem cell development.

Background of the Invention

Both B lymphocytes ("B cells") and T lymphocytes ("T cells") play important roles in mammalian immune systems. B cells control humoral immunity through antibody production. T cells account for nearly all forms of cellular immunity.

T cells originate in the thymus. Generally, thymic stem cells which are derived from precursor cells originating in bone marrow develop into blast cells which, in turn, develop into a variety of mature T cells. The developmental process from stem cell to mature T cell is referred to as stem cell differentiation or thymopoiesis. The developmental process from bone marrow precursor cell to mature T cell is referred to as T cell development. Stem cell development may include stem cell proliferation and differentiation into T cells. If stem cell differentiation progresses slowly or incompletely or if there is a shortage of stem cells in the thymus, a deficiency of T cells can result.

Thus, a T cell deficiency can result from a number of different factors. For example, T cell deficiencies exist in immunodeficient humans, such as individuals infected with HIV. In humans infected with HIV, it is believed that such deficiencies result not only from the virus attacking and killing T cells (e.g., CD4 cells) but also from a decrease in stem cell differentiation in the infected humans due to a change in the thymic milieu caused by the overall HIV infection. Moreover, T cell deficiencies exist in humans having a shortage of stem cells, such as individuals in need of a bone marrow transplant.

Thymosin Fraction Five (TF-5), originally described by Goldstein et al. (Proc. Nat'l Acad. Sci. (USA), 69:1800-1803 (1972)), is a partially purified extract of bovine thymus containing at least 40 peptide components, 20 of which have been purified to homogeneity or near homogeneity; it contains about 0.6% of Thymosin α_1 ($T\alpha_1$). Low, T.L.K., et al., "Thymosins: Structure, Function and Therapeutic Application", Thymus, 6:27-42 (1984).

Thymosin α_1 ("$T\alpha_1$") is a peptide originally derived from the thymus gland, which has been reported as containing 28 amino acids. Amino acid sequence information on $T\alpha_1$ is disclosed in U.S. Patent No. 4,079,127.

$T\alpha_1$ is an immune system modulator which heretofore has been reported as being useful, inter alia, in the treatment of lung cancer, Hepatitis B and Hepatitis C. Moreover, it previously has been suggested that $T\alpha_1$ might induce the expression of certain T cell markers as well as functional activity associated with lymphocyte maturation such as helper T cell activity, specific antidody production and production of

macrophage inhibiting factor. Low, 1984, supra, at page 33.

A T cell deficiency as described above can have devastating and sometimes fatal effects on those afflicted with this condition, as is evident from recent experience with AIDS. Clearly, there is a need in the art for new and effective methods of promoting development in humans who are T cell deficient. Accordingly, there is a need in the art for new and effective methods of promoting stem cell development in those individuals in need of such development.

Summary of the Invention

In accordance with the present invention, a method of promoting stem development in a mammal in need of stem cell development includes administering to said mammal a stem cell development enhancing effective amount of Thymosin α_1.

Also in accordance with the present invention, a method of promoting T cell differentiation from stem cells in a mammal in need of T cell differentiation includes administering to said mammal a T cell differentiation effective amount of Thymosin α_1.

Brief Description of the Drawings

Fig. 1 is a schematic diagram depicting the *in vitro* coculture model of thymopoiesis utilized in Example 3.

Fig. 2 is a graph depicting the results of purifying CD34+ stem cells from bone marrow. These results show that >99% of the stem cells utilized in

the *in vitro* coculture model of thymopoiesis expressed CD34+.

Fig. 3 depicts flow cytometry results which show the effect which $T\alpha_1$ had on the cell number per well as a function of time in the *in vitro* stem cell-cultured thymic epithelial fragment (SC-CTEF) coculture model of thymopoiesis illustrated in Fig. 1.

Fig. 4 depicts flow cytometry results which show the effect $T\alpha_1$ had on the expression of CD25+ as a function of time in the *in vitro* SC-CTEF coculture model of thymopoiesis illustrated in Fig. 1.

Fig. 5 depicts flow cytometry results which show the effect $T\alpha_1$ had on the expression of CD44+25+ as a function of time in the *in vitro* SC-CTEF coculture model of thymopoiesis illustrated in Fig. 1.

Fig. 6 depicts flow cytometry results which show the effect $T\alpha_1$ had on the expression of CD44+25+3- as a function of time in the *in vitro* SC-CTEF coculture model of thymopoiesis illustrated in Fig. 1.

Fig. 7 depicts flow cytometry results which show the effect $T\alpha_1$ had on the expression of single positive CD4+ (spCD4+) as a function of time in the *in vitro* coculture model of thymopoiesis illustrated in Fig. 1.

Description of the Preferred Embodiments

It surprisingly has been discovered that Thymosin α_1 ($T\alpha_1$) is particularly and unexpectedly effective in promoting stem cell development. This is particularly advatageous for mammals in need of such development.

The terms "Thymosin α_1" and "Tα_1" refer to peptides having the amino acid sequence disclosed in U.S. Patent No. 4,079,127, the disclosure of which is incorporated herein by reference.

According to the present invention, methods of promoting stem cell development and T cell differentation from stem cells are provided. The methods of the present invention include administering to the mammal a stem cell development enhancing effective amount of Tα_1.

According to preferred embodiments of the present invention, effective amounts of Tα_1 are administered to mammals in need of stem cell development to promote stem cell development in the mammals. The amount of Tα_1 administered to the mammals to promote stem cell development is preferably between about 0.1 μg Tα_1 per kg body weight of the mammal and about 4 μg Tα_1 per kg body weight and, more preferably, between about 5 μg Tα_1 per kg body weight (i.e., about 0.5 mg Tα_1) and about 65 µg Tα_1 per kg body weight (i.e., about 5 mg Tα_1). In a most preferred embodiment of this invention, about 20 µg Tα_1 per kg body weight (i.e., about 1.6 mg_Tα_1) is administered to the mammals in need of stem cell development. Actual dosages administered can be in the range of about 0.1-10 mg, about 0.5 - 5 mg or about 1-2 mg, with a dosage of 1.6 mg being contemplated. In these embodiments, the mammals preferably are human.

According to one aspect of the present invention, the mammals to which Tα_1 is administered are in need of stem cell development. Mammals in need of stem cell development may include, but are not limited to, mammals that are T cell- and/or stem cell-deficient.

$T\alpha_1$ may also promote stem cell proliferation. Mammals afflicted with an immunodeficiency disease (e.g., humans who have AIDS or are HIV positive) and animals in need of a bone marrow transplant are generally in need of stem development. According to another aspect of the present invention, $T\alpha_1$ can be administered to mammals in need of stem cell development on a routine basis. For example, $T\alpha_1$ can be administered daily, twice a day, three times a day, every other day, weekly, monthly, etc. The frequency of administration, of course, will depend upon the quantity and formulation of $T\alpha_1$ being administered.

According to the preferred embodiments of the present invention, compositions containing $T\alpha_1$ may be formulated in a conventional manner for administration by any suitable route. Suitable routes of administration include, but are not limited to, oral, rectal, nasal, topical, vaginal, and parenteral (including subcutaneous, intramuscular, intravenous and intradermal). Particularly preferred embodiments utilize oral or parenteral administration, with parenteral administration being a more preferred embodiment. It will be appreciated that the preferred route may vary with the condition, age and species of the recipient.

While not essential, in preferred embodiments, $T\alpha_1$ is administered as part of a pharmaceutical formulation. The formulations of the present invention comprise $T\alpha_1$ together with one or more pharmaceutically acceptable carriers and optionally with other therapeutic ingredients. The carrier(s) are "acceptable" in the sense of being compatible with the

other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form, e.g., tablets and sustained release capsules, and may be prepared by any suitable pharmaceutical methods.

Such methods include, but are not limited to, the step of bringing into association $T\alpha_1$ with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association $T\alpha_1$ with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of $T\alpha_1$; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion, etc.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine $T\alpha_1$ in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be

formulated so as to provide slow or controlled release of the active ingredient therein.

Formulations suitable for topical administration include lozenges comprising $T\alpha_1$ in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising $T\alpha_1$ in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising $T\alpha_1$ to be administered in a suitable liquid carrier.

Formulations suitable for topical administration to the skin may be presented as ointments, creams, gels and pastes comprising $T\alpha_1$ and a pharmaceutically acceptable carrier, or may utilize a transdermal patch containing the ingredient to be administered.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for nasal administration wherein the carrier is a solid include a coarse powder having a particle size, for example, in the range from about 20 to about 500 microns which is administered in the manner in which snuff is taken, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid, for administration, as for example, a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Formulations suitable for vaginal administration may be presented as tampons, creams, gels, pastes, foams or spray formulations containing, in addition to $T\alpha_1$, suitable carriers.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile

injection solutions which may optionally contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other suitable agents having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

The present invention is applicable to native (i.e., naturally occurring) $T\alpha_1$, as well as synthetic $T\alpha_1$ and recombinant $T\alpha_1$ having the amino acid sequence of native $T\alpha_1$, amino acid sequences substantially similar thereto, or an abbreviated sequence form thereof, and their biologically active analogs (including muteins) having substituted, deleted, elongated, replaced, or otherwise modified sequences which possess bioactivity substantially similar to that of $T\alpha_1$.

The following examples are for illustrative purposes only, and are not to be construed in a limiting sense.

Examples

EXAMPLE 1

Preparation of Injectable Formulation

Pharmaceutical dosage units of 1 ml each are prepared from the ingredients shown in Table 1 below.

TABLE 1

Active Ingredient	Amount Per mL
Thymosin α-1	0.0064 g
Inactive Ingredients	
mannitol, U.S.P.	0.050 g
sodium phosphate dibasic, heptahydrate, U.S.P.	0.002 g
sodium phosphate monobasic, monohydrate, U.S.P.	0.0005 g
sodium phosphate dibasic, 2 mg/ml solution	
sodium phosphate monobasic, 0.5 mg/ml solution	
water for injection, U.S.P.	

EXAMPLE 2

Preparation of Injectable Formulation

Pharmaceutical dosage units of 1 ml each are prepared from the ingredients shown in Table 2 below.

TABLE 2

Active Ingredient	Amount Per mL
Thymosin α-1	0.0032 g
Inactive Ingredients	
mannitol, U.S.P.	0.050 g
sodium phosphate dibasic, heptahydrate, U.S.P.	0.002 g
sodium phosphate monobasic, monohydrate, U.S.P.	0.0005 g
sodium phosphate dibasic, 2 mg/ml solution	
sodium phosphate monobasic, 0.5 mg/ml solution	
water for injection, U.S.P.	

EXAMPLE 3

Effects of Thymosin-α_1 on CD34+ Stem Cell Differentiation in Cultured Thymic Epithelia *in vitro*

Material and Methods

To evaluate the effect of Tα_1 on thymopoiesis, an *in vitro* coculture model of thymopoiesis was utilized. In this model, as set forth in Figure 1, CD34+ stem cells were isolated from bone marrow by positive selection using CD34+ antibody coated beads and immunomagnetic selection, MiniMACS (Miltenyi). As illustrated in Figure 2, the separation procedure was extremely efficient with >99% of the stem cells expressing CD34+. CD34+ stem cells were a heterogeneous population. Thus, in some experiments, more immature CD34+ population was used, depleted of CD2+CD4+ cells.

Thymus tissue was obtained from children less than 2 years of age undergoing cardiac surgery. The thymic tissue was minced into 1 mm^3 fragments and cultured in 1.35 mM 2-deoxyguanosine for 10-14 days in order to deplete the thymic tissue of thymocytes in a 5% CO_2 humidified atmosphere. The thymic fragments were then cultured in growth media (Iscove's/Ham's F-12 media supplemented with 5% FCS and epidermal growth factor).

Subsequently, CD34+ stem cells were cocultured with the cultured thymic epithelia fragments in 24-well transwell culture plates in Iscove's/Ham's F-12 media supplemented with 5% FCS, epidermal growth factor and IL-2 in a 5% CO_2 humidified atmosphere. Approximately 100,000 CD34+ stem cells were cocultured with 3-5 thymic fragments. 10 μg/ml of Tα_1 was added to parallel

cocultures. Media and $T\alpha_1$ of cocultures were replaced twice weekly.

At weekly intervals, cultures were harvested to analyze thymocyte subpopulations by flow cytometry. T cell surface phenotypes of the differentiated stem cells were determined by reacting monoclonal antibodies (mAb) conjugated with either fluorescein (FITC), phycoerythrin (PR), and peridinin chlorophyll protein (Per-CP) and were then analyzed by flow cytometry. Monoclonal antibodies used are listed in Table 3 below. Combinations of mAbs were used to identify immature thymocytes, such as double-positive (CD3-CD4+CD8+) and triple positive (CD3+CD4+CD8+) thymocytes, and mature CD3+C4+CD8- and CD3+CD4+CD8+ T cells. Analysis of thymocyte subpopulations was performed using WinList software.

Results

Initially, a dose response of $T\alpha_1$ was performed ranging from 1 to 10 μg/ml. Optimal effects were observed at 10 μg/ml which then was used in subsequent experiments. As shown in Figure 3, $T\alpha_1$ increased the proliferation of cocultured cells harvested at all time points from the cocultures compared to media alone. Increased proliferation or expansion of thymocytes stimulated by $T\alpha_1$ paralleled media alone with peak proliferation observed at 3 weeks, 16.6 versus 10.4 fold increase.

Thymocyte subpopulations were then analyzed to determine whether $T\alpha_1$ affected thymocyte maturation. As illustrated in Figure 4, $T\alpha_1$ significantly increased thymocyte CD25+ expression at 1, 3 and 4 weeks of coculture. $T\alpha_1$ significantly stimulated CD25+

expression at 1 week, 40% versus 23%, and was significantly increased at 4 weeks, 56% versus 31%.

CD44 and CD25 expression were also evaluated in this model system since CD44 expression appears early in differentiation in the subcapsular cortex prior to expression of TCR, CD3, CD4 and CD8, i.e. triple negative thymocytes. Other groups have reported that CD44 plays an important role in thymocyte migration and maturation. As thymocytes further mature through double and triple positive thymocyte stages, CD44 disappears but then reappears in more mature thymocytes. The ligands for CD44 include hyaluronate and fibronectin, matrix proteins which promote cell adhesion. In this model of stem cell differentiation, $T\alpha_1$ significantly increased CD44+CD25+ expression in the first three weeks of coculture, peaking at 3 weeks 46% versus 30%. See Figure 5. Furthermore, $T\alpha_1$ increased early thymocyte maturation by increased expression of CD44+25+3- thymocytes at 1 week, 12% versus 7%. See Figure 6. Though $T\alpha_1$ did not affect CD3, CD4 CD8 expression, it did increase mature single positive CD3+4+ expression at 3 weeks, 31% versus 23%. Other thymocyte surface phenotypes, such as TCR, CD2, CD7, CD45RA and CD45RO, were not affected by $T\alpha_1$.

Thus, $T\alpha_1$ dramatically increased CD25 expression throughout the coculture period. It stimulated expression of an early thymocyte, CD44+25+3- pre-T cell, at 1 week of coculture. As thymocytes matured, expression of this thymocyte stage waned similar to control cocultures. $T\alpha_1$ also promoted expression of mature single positive CD4+ T cells at 3 weeks of coculture. Throughout the coculture period, $T\alpha_1$ stimulated proliferation of thymocytes. From these

studies it is clear that $T\alpha_1$ promotes T cell differentiation from stem cells.

TABLE 3

Monoclonal antibodies

mAb	Clone	Specificity
		Mononuclear Cells
CD7	Leu9	Prethymic Precursor T cell
CD33	LeuM9	Progenitors, monocytes; related to myelin-associated glycoprotein
CD34	HPCA-2	Bone marrow progenitor cell
CD38	Leu17	Progenitors; growth-factor receptor
CD44	Leu44	Progenitor cell committed to thymocytes and thymic dendritic cells
CD2	Leu5b	T and NK cells; cytoadhesion molecule binding LFA-3
CD3	Leu4	Multichain receptor associated with TCR
CD4	Leu3a	T helper/inducer; cytoadhesion glycoprotein binds to MHC II
CD8	Leu2a	T cytotoxic/suppressor; cytoadhesion glycoprotein binds to MHC I
TCRαβ	WT31	T cell receptor α/β chains
TCRγδ	11F2	T cell receptor γ/δ chains
CD25	2A3	Low affinity IL-2 α chain receptor
CD69	Leu23	B- and T cells; activation-inducer
CD45RA	Leu18	Isoform of CD45; naive cell
CD45RO	UCHL-1	Isoform of CD45; memory cell
CD14	LeuM3	Monocytes, dendritic cells
CD49d	HP2.1	VLA-α4 integrin chain, fibronectin receptor
CD49e	SAM1	VLA-α5 integrin chain, fibronectin receptor
CD11a	25.3	LFA-1 integrin αL chain
CD18	7E4	Integrin β2 chain
CD29	K20	Integrin β1 chain
CD16	Leu11c	Natural killer, granulocytes; Fcγ RIII
CD56	Leu19	Isoform of N-CAM; NK and T cytotoxic subset
CD20	Leu16	B cells
HLA-DR	DR	HLA cells class II molecules

mAb	Clone	Specificity
		Thymic Epithelia
TE3	7B12	Cortical thymic epithelium and fibroblast
TE4	TE4	Medullary and subcapsular cortical thymic endocrine epithelium
CDR2		pan epithelia
		HIV
p24	26C	HIV-1 p24, cytoplasmic
Protease	H2930	HIV-1 protease

EXAMPLE 4

Treatment of Adults in Need of Stem Cell Differentiation With $T\alpha_1$

Adult patients in need of stem cell differentiation are selected (e.g., adults with a T cell count of less than 200).

Each patient receives $T\alpha_1$ at a dose of 1.6 mg subcutaneously (SQ) every other day.

Outpatient following is initially at one-week intervals for two weeks, then at two-week intervals for two months, and then monthly for the remainder of the treatment period. At each visit the T cell count is monitored.

Drug toxicity is monitored on an ongoing basis using both clinical and laboratory parameters.

While the invention has been described and illustrated with details and references to certain preferred embodiments, those skilled in the art will appreciate that various modifications, changes,

omissions, and substitutions can be made without departing from the spirit of the invention.

What is claimed is:

1. A method of promoting stem cell development in a mammal in need of stem cell development which comprises administering to said mammal a stem cell development enhancing effective amount of Thymosin α_1 ($T\alpha_1$).

2. The method of claim 1, wherein said mammal is HIV positive.

3. The method of claim 1, wherein said mammal is in need of a bone marrow transplant.

4. The method of claim 1, wherein the amount of $T\alpha_1$ administered to said mammal is between about 0.1 μg $T\alpha_1$ per kg body weight of the mammal and about 4.0 μg $T\alpha_1$ per kg body weight.

5. The method of claim 4, wherein the amount of $T\alpha_1$ administered to said mammal is between about 5 μg $T\alpha_1$ per kg body weight of the mammal and about 65 μg $T\alpha_1$ per kg body weight.

6. The method of claim 5, wherein the amount of $T\alpha_1$ administered to said mammal is about 20 μg $T\alpha_1$ per kg body weight of the mammal.

7. The method of claim 1, wherein said mammal is human.

8. The method of claim 1, comprising promoting T cell differentiation from said stem cells.

9. The method of claim 1, wherein said amount is a dosage of about 0.1-10 mg.

10. The method of claim 1, wherein said amount is a dosage of about 0.5-5 mg.

11. The method of claim 1, wherein said amount is a dosage of about 1-2 mg.

12. The method of claim 1, wherein said amount is a dosage of about 1.6 mg.

FIG. 1

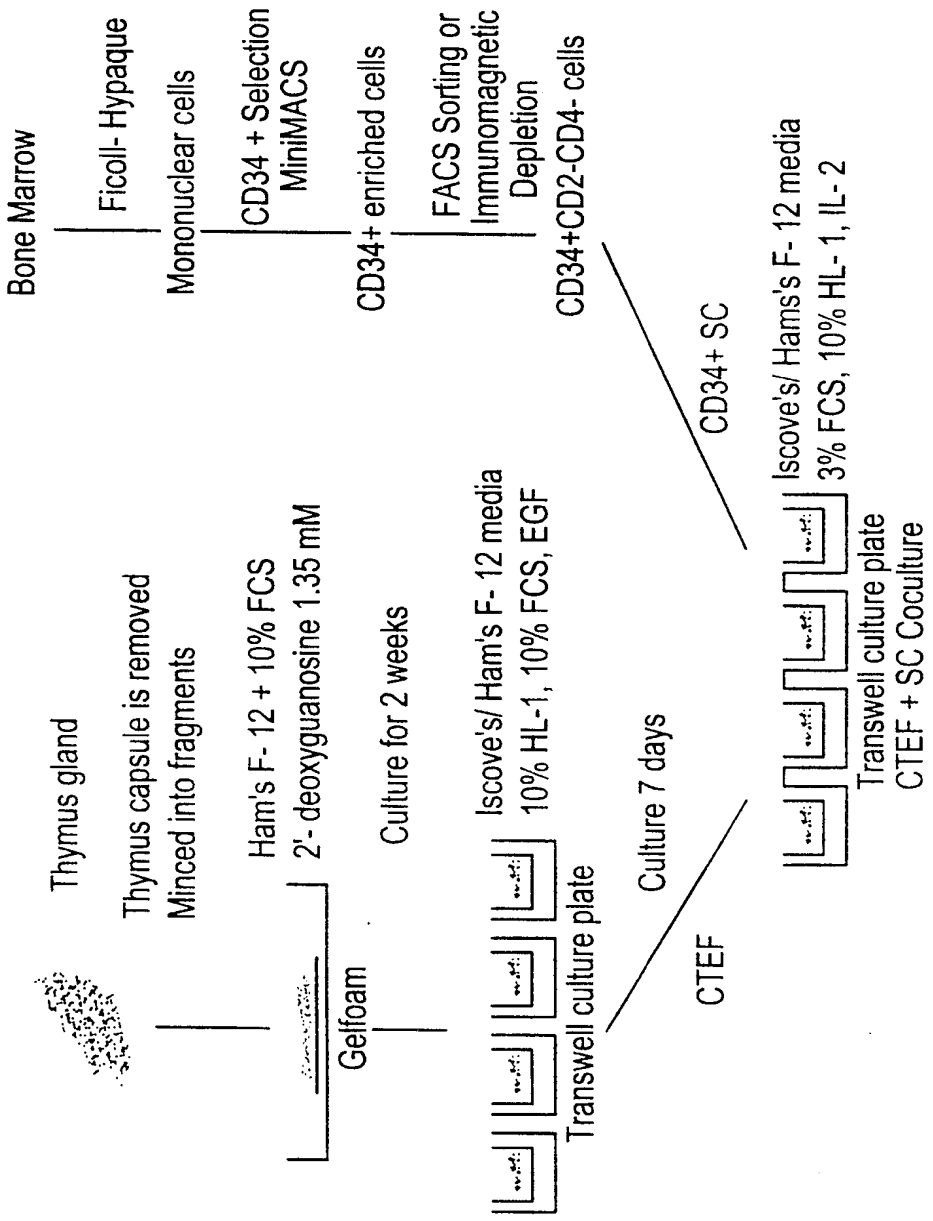

Thymus gland
Thymus capsule is removed
Minced into fragments
Ham's F- 12 + 10% FCS
2'- deoxyguanosine 1.35 mM
Gelfoam
Culture for 2 weeks
Iscove's/ Ham's F- 12 media
10% HL-1, 10% FCS, EGF
Transwell culture plate
Culture 7 days
CTEF
Bone Marrow
Ficoll- Hypaque
Mononuclear cells
CD34 + Selection
MiniMACS
CD34+ enriched cells
FACS Sorting or
Immunomagnetic
Depletion
CD34+CD2-CD4- cells
CD34+ SC
Iscove's/ Hams's F- 12 media
3% FCS, 10% HL- 1, IL- 2
Transwell culture plate
CTEF + SC Coculture

FIG. 2

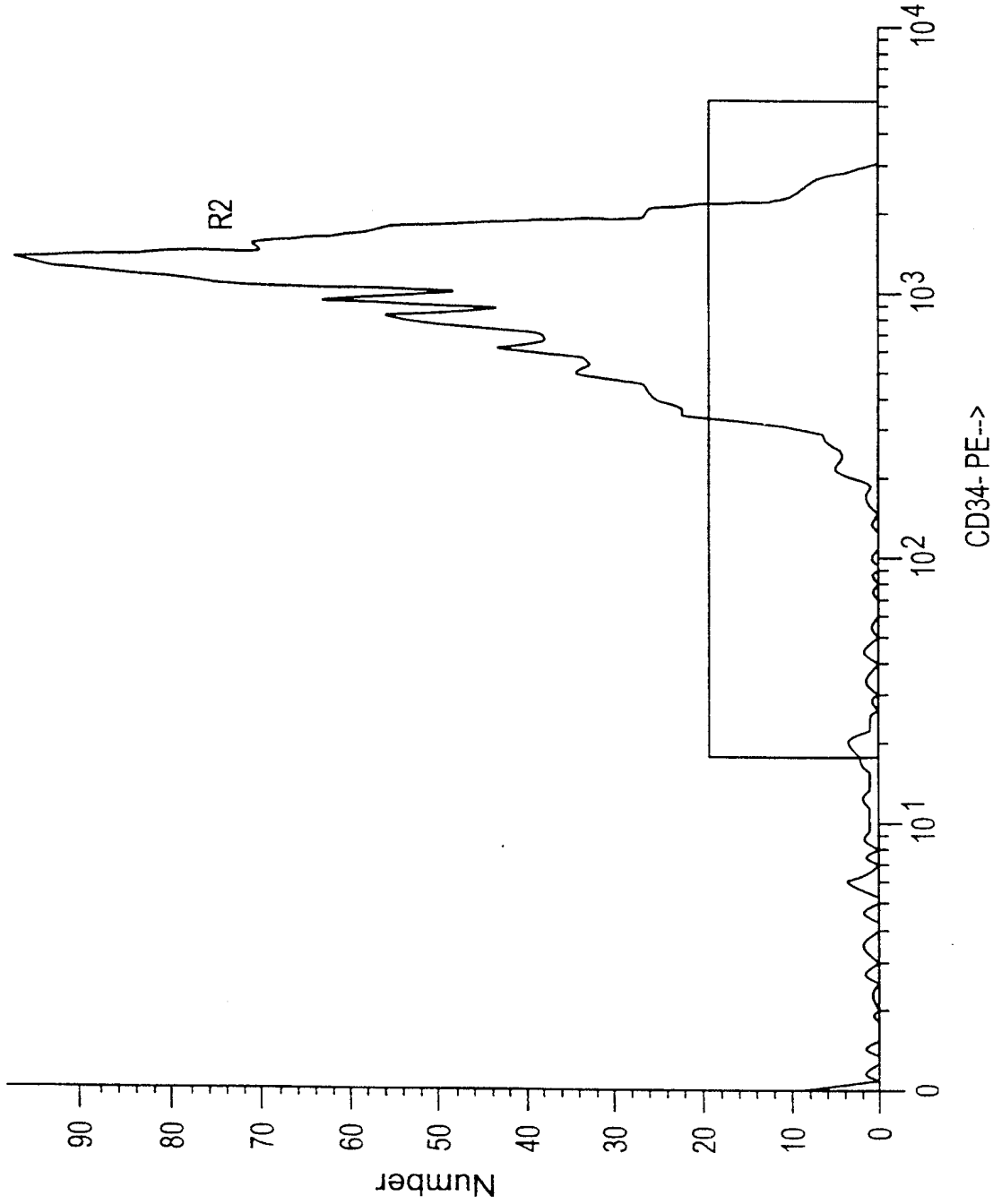


FIG. 3

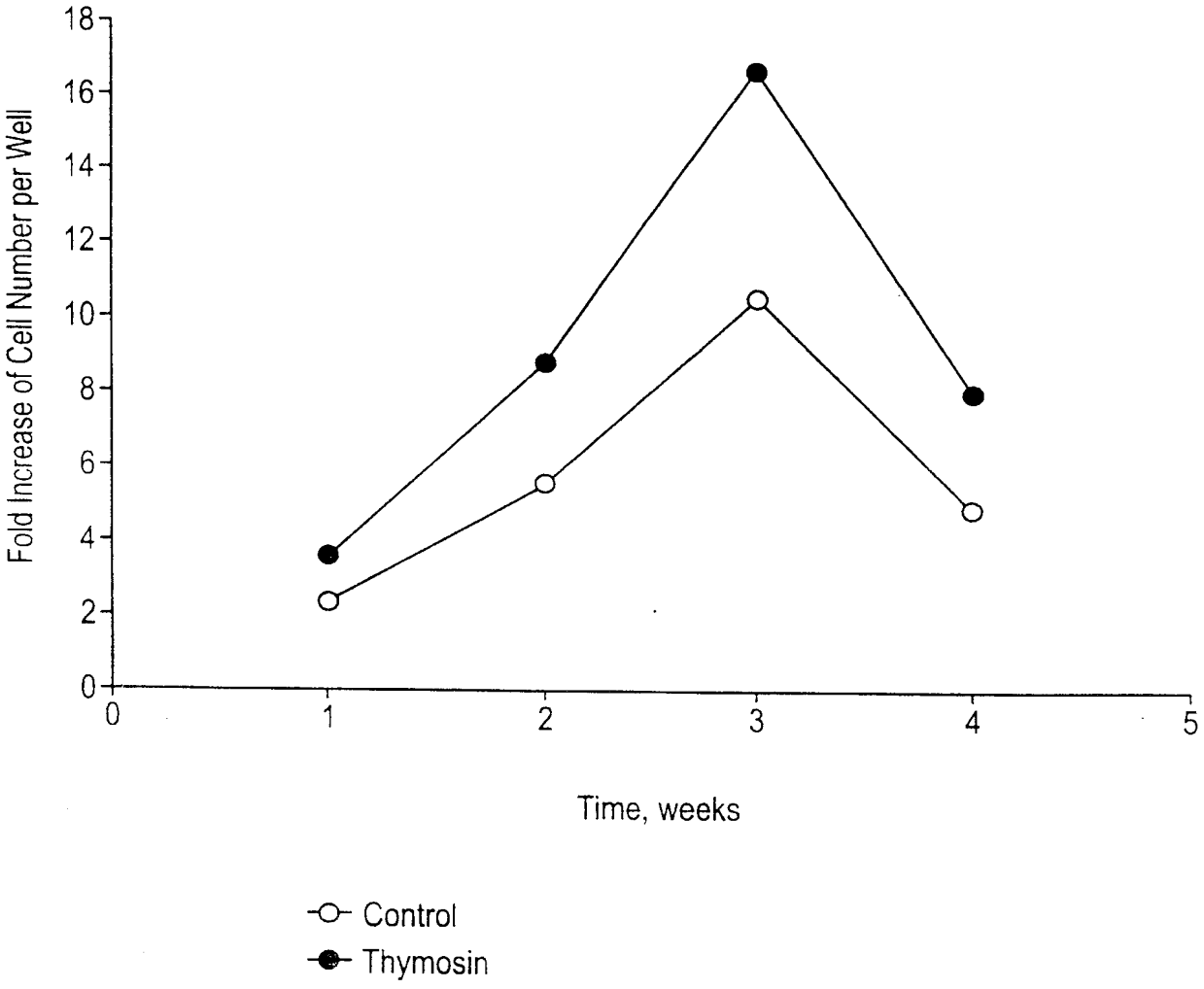
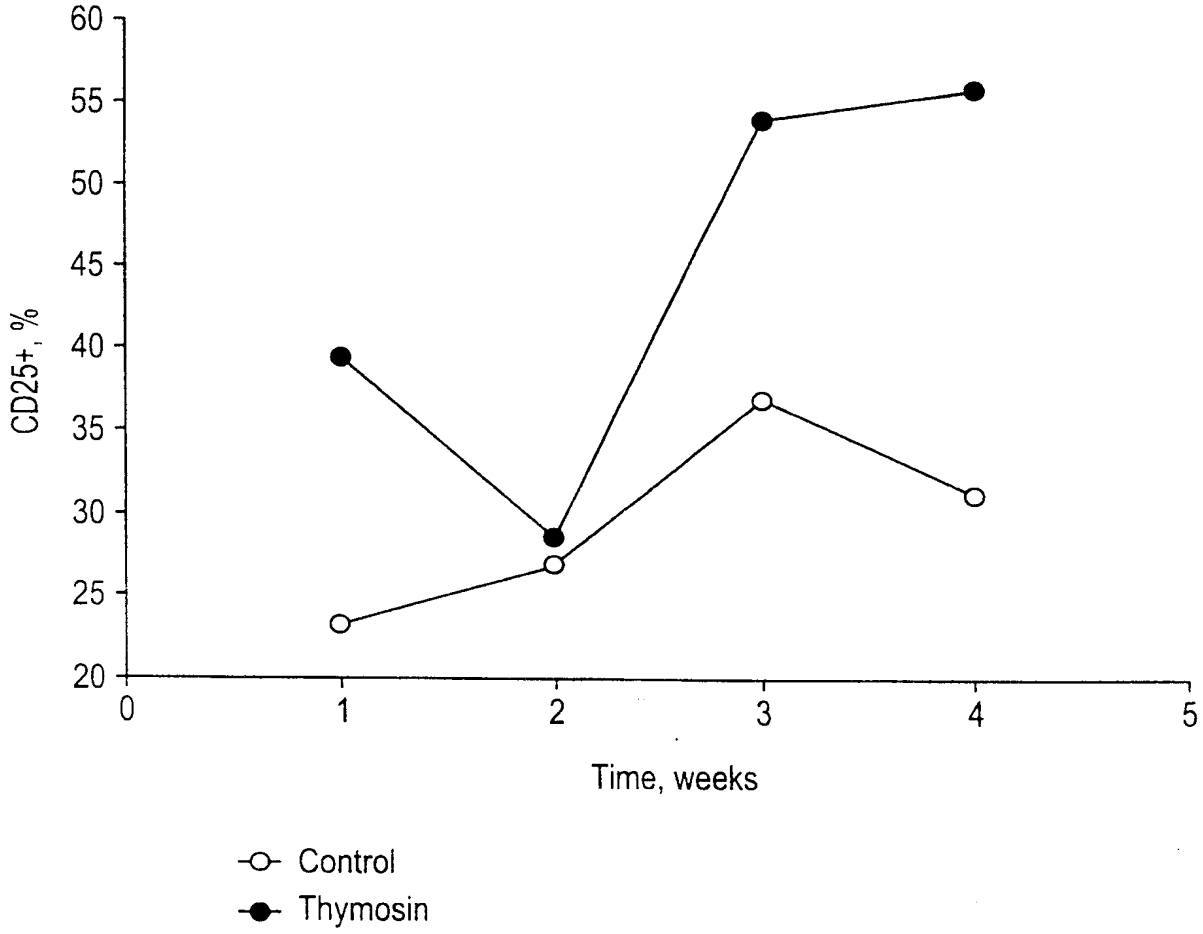

Fold Increase of Cell Number per Well
18
16
14
12
10
8
6
4
2
0
0
1
2
3
4
5
Time, weeks
Control
Thymosin

FIG. 4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CD25+, %
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4
5
Time, weeks
Control
Thymosin

FIG. 5

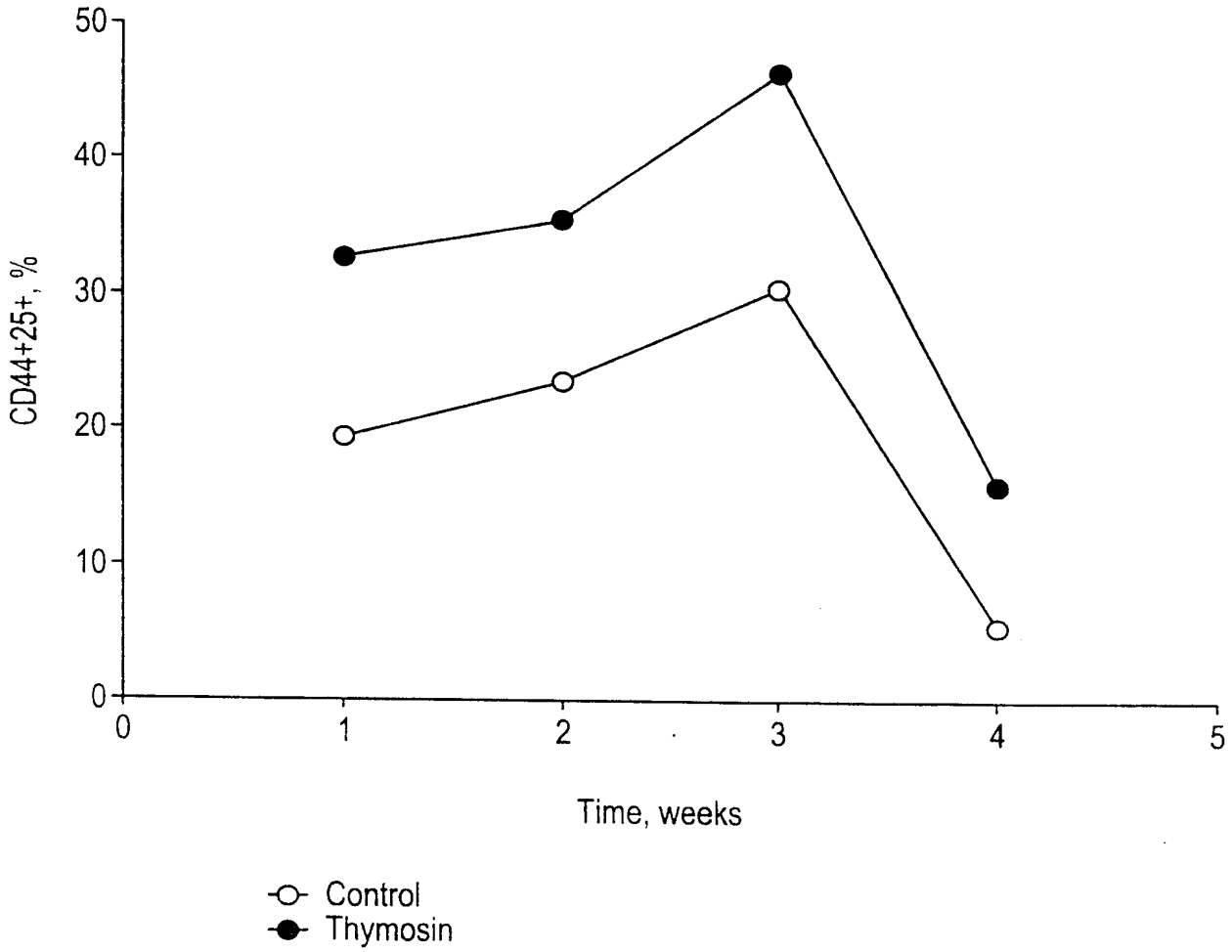


FIG. 6

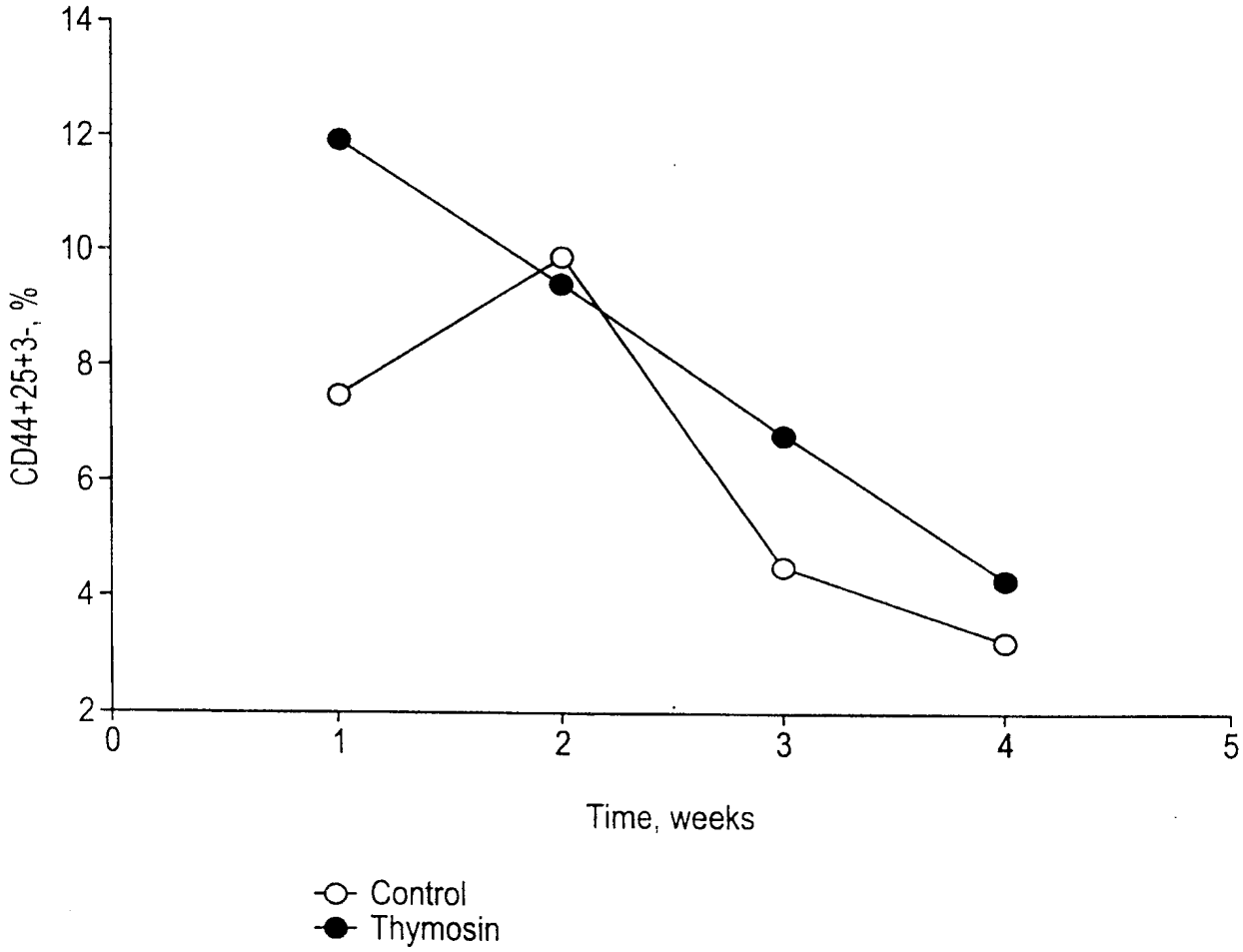
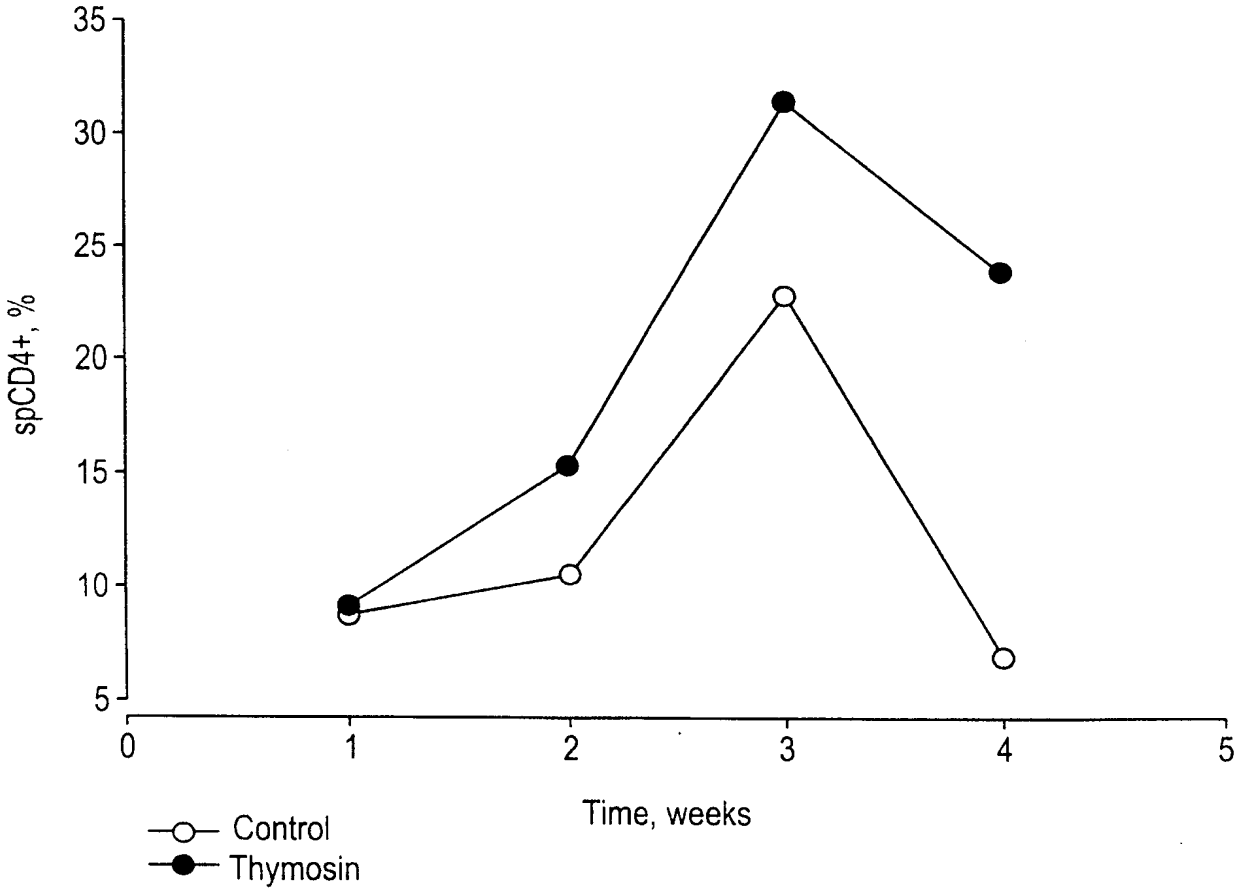


FIG. 7



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/02830

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YUMIKO OHTA ET AL.: "Immunomodulating activity of thymosin fraction 5 and thymosin alpha 1 in immunosuppressed mice" CANCER IMMUNOL. IMMUNOTHER., vol. 15, no. 2, 1983, pages 108-113, XP002063754	1
Y	see abstract	2-12
X	YUMIKO OHTA ET AL.: "Thymosin alpha 1 enhances haematopoietic colony formation by stimulating the production of interleukin 3 in nu/nu mice" INT.J.IMMUNOPHARMACOL., vol. 8, no. 7, 1986, pages 773-779, XP002063755	1
Y	see abstract see page 777 - page 778	2-12

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 April 1998	22 05. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Tzschoppe, D

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/02830

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	CHEMICAL ABSTRACTS, vol. 92, no. 3, 21 January 1980 Columbus, Ohio, US; abstract no. 16153, KHAITOV ET AL.: "Factors controlling hematopoietic stem cell recirculation. IV. Effect of thymosin on the migration and differentiation of hematopoietic stem cells" XP002063757 see abstract & TSITOLOGIYA, vol. 21, no. 9, 1979, pages 1058-1064, ---	1-12
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Y	see abstract & NAGOYA-SHIRITSU DAIGAKU IGAKKAI ZASSHI, vol. 41, no. 3, 1990, pages 467-482, ---	2-12
P,X	AP KNUTSEN ET AL.: "In vitro effect of thymosin-alpha 1on stem cell differentiation in cultured thymic epithelia" JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, vol. 99, no. 1 part 1, January 1997, page s131 XP002063756 see abstract -----	1,8

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